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Towards an Understanding of Physiological and Biochemical Mechanisms of Drought Tolerance in Plant

Yogendra K. Meena^{1*} and Nirmaljit Kaur²

¹*Department of Vegetable Science, Punjab Agricultural University, Ludhiana, Punjab 141-004, India.*

²*Department of Botany, Punjab Agricultural University, Ludhiana, Punjab 141-004, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Author YKM managed the literature searches and wrote the first draft of the manuscript. Author NK critically review the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Drought stress is one of the major abiotic stress that can causes huge loss to the world food production. It remains a major contributor to severe food shortage and famine. With a consistent increase in world population, pressure will continue to mount on the existing yet limited water resources. The situation is respected to further aggravate due to the predicted increase in temperature and decrease in precipitation consequent upon global warming. Water scarcity has already become a severe constraint in plant survival and productivity of crops in arid and semi-arid regions. The active response of plants to drought stress through various biochemical and physiological modifications improves the metabolism and can further the mobilize various defense mechanisms in order to enhance survival of the plants under conditions of drought. In this review, various physiological and biochemical responses in plants towards enhancement of drought tolerance are discussed.

*Corresponding author: E-mail: yogendra-coavs@pau.edu;

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1. INTRODUCTION

Drought is one of the most significant abiotic stress that limits the growth and productivity of crop plants [1]. Drought triggers a wide array of plant responses, ranging from cellular metabolism to changes in growth rates and crop yields. Exposure of plants to extreme stress conditions such as drought initiate a diverse set of physiological, morphological and developmental changes in order to survive in adverse condition, as has been widely reported [2,3]. However, tailoring crops in under to enable them to grow successfully in environments that are drought prone is promising [4]. Drought stress progressively decreases CO₂ assimilation rates due to reduced stomatal conductance. It reduces leaf size, stem extension and root proliferation; disturbs plant water relations and reduces water-use efficiency. It disrupts the photosynthetic pigments and reduces the gas exchange leading to a reduction in plant growth and productivity. The critical role of osmolyte accumulation under drought stress conditions has been researched actively to understand the tolerance of plants to dehydration [4]. Several mechanisms have been adopted by drought tolerant plants to adapt to water stress including reduction in water loss by increasing stomatal resistance, increase of water uptake by developing large and deep root system and accumulation of osmolytes [5]. The accumulated osmolytes are amino acids such as proline, glutamate, glycine-betaine and sugars (mannitol, sorbitol and trehalose). These compounds play a key role in preventing membrane disintegration and enzyme inactivation in the low water available environment. Plants display a variety of physiological and biochemical responses at cellular and whole-organism level towards prevailing drought stress, thus making it a complex phenomenon. The identification of suitable plant characters for screening large number of genotypes in a short time at critical stage of crop growth with the aim of selecting drought tolerant cultivars remains a major challenge to the plant breeder [4,6].

The measurement of solute leakage from plant tissue is a proven method for measuring membrane integrity in relation to environmental stress [7]. This technique involves measurement of electrolyte leakage into an aqueous medium, where the degree of cell membrane stability is considered to be one of the best physiological

indicators of drought stress tolerance [8]. Maintenance of turgor pressure during stress is important to preserve metabolic response in crop species and is a well recognized mechanism in breeding toward drought stress tolerance. Relative water content (RWC) allows for the comparison of metabolic changes in the plant, at the same cellular water status. RWC further allows the estimation of plant water status in terms of cellular hydration and is under the possible effect of both leaf water potential and osmotic adjustment. RWC can be used effectively to evaluate drought tolerance and selection of the most drought tolerant genotypes [9]. Plants have evolved a number of enzyme and non-enzyme antioxidants that ameliorate oxidative stress by scavenging reactive oxygen species (ROS). Drought stress causes oxidative injury, and the ability to increase the levels of antioxidative capacity or increased levels of antioxidants during stress can limit membrane damage and enzyme activity can be an important measurement of drought tolerance There are numerous reports on these enzymes protecting plants during oxidative stress initiated by drought [5,6,10,11].

Although plant stress tolerance mechanisms are not known clearly, accumulation of new proteins and stress gene expression that encode biosynthetic enzymes against osmotic stress have been investigated. Also a study on gene proteins have shown that osmotic proteins increase in low water stress [12]. The quantity of aquaporin existing in plasma membrane regulates membrane hydrolytic function and increases water permeability under environmental factors like low water, hormones and light conditions.

2. PLANTS ARE CATEGORIZED BROADLY DEPENDING UPON THEIR RESPONSE TO DROUGHT

2.1 Drought Escape

Drought escape is defined as the ability of a plant to complete its life cycle before serious soil and plant water deficits occurs. This mechanism involves rapid phenological development (early flowering and early maturity), developmental plasticity (variation in duration of growth period depending on the extent of water deficit) and remobilization of pre anthesis assimilates [13].

2.2 Drought Avoidance

Drought avoidance comprises of mechanisms that reduce water loss from plants, by stomatal control of transpiration, and also maintain water uptake through an extensive and prolific root system [14]. Hence, drought avoidance involves minimizing water loss (closing stomata, reducing light absorbance through rolled leaves, decreasing canopy leaf area) and maximizing water uptake (increasing investment in the root, reallocation of nutrients stored in older leaves, higher rates of photosynthesis) [15].

2.3 Drought Tolerance

Drought tolerance is the ability of plants to withstand water-deficit with low tissue water potential. The mechanism of drought tolerance is maintenance of turgor through osmotic adjustment (accumulation of solutes in the cell), increased cell elasticity & decreased cell size and desiccation tolerance by protoplasmic resistance [13].

3. PHYSIOLOGICAL AND BIOCHEMICAL RESPONSE

Drought stress causes tissue dehydration which is characterized by fundamental changes in water relations, physiological and biochemical processes, membrane structure as well as ultrastructure of subcellular organelles. At the whole-plant level, drought stress leads to a progressive suppression of photosynthesis caused by stomatal and non-stomatal limitations. Tolerant genotypes not only retain sufficient water under drought, but also have a highly active system for protection against oxidative stress injury.

3.1 Photosynthetic Pigments

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing power [16]. Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation as reported in cherry tomato [17]. The Chlorophyll a and b ratio reduced in resistant species of tomato against low water condition and this indicated that photosystem II protects

the plant against low water stress [18]. Drought stress is known to inhibit photosynthetic activity in tissues due to an imbalance between light capture and its utilization [4]. The decrease in the maximum quantum yield of PSII photochemistry FV/FM (Variable fluorescence: Maximum fluorescence) implies a decrease in the capture and conversion rate of excitation energy by PSII reaction centre and so, a reduction in PSII photochemical efficiency indicating the disorganisation of PS II reaction centres under water stress conditions. In general, the harvested energy in excess of that consumed by the Calvin Cycle must be dissipated to avoid oxidative stress which leads to decrease PSII performance. FV/FM was not affected by drought in *Calluna*, but a small (1.5%) yet significant decrease was seen in *Deschampsia* across season. Photosystem II (PSII) is highly sensitive to light and down regulation of photosynthesis under drought stress causes an energy imbalance in the PSII reaction centre leading to photo-inhibition [19]. Mechanisms have evolved in the plant to protect from photo-inhibition, such as non photochemical quenching, transport to molecules other than CO₂, particularly to oxygen, which leads to photorespiration and Mehler reaction [20], non-radiative energy dissipation mechanisms [21] and chlorophyll concentration changes [19].

3.2 Stomatal Conductance

One of the basic mechanisms for reducing the impact of drought is early stomatal closure at the beginning of the period of water deficit. Stomatal closure not only reduce water loss, but also reduce the gas exchange between the plant and the ambient air. The reduced CO₂ intake then results in reduced photosynthesis [22]. As plant water potential falls due to water deficit, photosynthesis rate reduces. Water deficit causes reduction in photosynthesis mainly due to decreased stomatal conductance. Stomatal closure has been reported in tomato at leaf water potential (leaf) between -0.7 to 0.9 MPa [23], in pepper at -0.58 to -0.88 MPa [24], however eggplant can withstand a greater drought than the most other vegetables. Bahadur et al. [25] observed significant reduction in photosynthesis rate and stomatal conductance in spring-summer okra when water stress was imposed for 10 or 12 days. Although stomatal conductance is the major limitation to photosynthesis under drought conditions in cowpea; however, a pronounced non-stomatal limitation may occur under severe drought stressed conditions that may also lead to

impairment of photosynthetic activity [26]. If perpetual decline in photosynthesis is more than the transpiration, then non-stomatal factors contribute more to the reduction of photosynthesis than the stomatal effects. This is because stomatal resistance accounts for a smaller portion of total resistance in CO₂ pathway. During this non-stomatal control of photosynthesis, intercellular resistance for CO₂ from the intercellular space of the chloroplasts plays an important role. Thus, a decrease of the photosynthetic rate under water deficit condition can be attributed to both stomatal and non-stomatal limitations. Non-stomatal photosynthesis limitation has been attributed to the reduced carboxylation efficiency, ribulose-1,5-bisphosphate (RuBP) regeneration, amount of functional Rubisco, or to the inhibited functional activity of PSII. The drought-tolerant species control stomatal function to allow some carbon fixation during stress, thus improving water use efficiency, or open stomata rapidly when water deficit is relieved. In fact, stomatal conductance can be used as an integrative parameter to reflect the severity of drought stress.

3.3 Cell Membrane Stability

A major impact of plant environmental stress is cellular membrane modification, which results in its perturbed function or total dysfunction. The cellular membrane dysfunction due to stress is expressed by increased permeability and leakage of ions, which can be readily measured by the efflux of electrolytes, and may be used as a tolerance index for drought stress [27]. It is generally accepted that the maintenance of integrity and stability of membranes under water stress is a major component of drought tolerance in plants [28]. Cell membrane stability declined rapidly in Kentucky bluegrass when exposed to drought. Cell membrane stability, reciprocal to cell membrane injury is a physiological index widely used for the evaluation of drought tolerance. Moreover, it is a genetically related phenomenon since quantitative trait loci for this have been mapped in drought-stressed rice at different growth stages. Dhanda et al. [29] showed that membrane stability of the leaf segment was the most important trait to screen the germplasm for drought tolerance. The increase in cell membrane stability under water deficit conditions in response to drought stress is species specific and correlation with a reduction in relative growth rate. In holm oak (*Quercus ilex*) seedlings, hardening increased drought tolerance primarily by reducing osmotic potential

and stomatal regulation, improved new root growth capacity and enhanced cell membrane stability. Among treated seedlings, the greatest response occurred in seedlings subjected to moderate hardening. Variation in cell membrane stability, stomatal regulation and root growth capacity was negatively related to osmotic adjustment [30].

3.4 Relative Water Content

Relative water content (RWC) is related to water uptake by the roots as well as water loss by transpiration. A decrease in the RWC in response to drought stress has been noted in wide variety of plants as reported by Nayyar and Gupta [31] that leaves when subjected to drought, exhibit large reductions in RWC and water potential. Exposure of plants to drought stress substantially decrease the leaf water potential, relative water content and transpiration rate with a concomitant increase in leaf temperature. RWC is affected by the interaction of severity, duration of the drought and species [4,32]. Bahadur et al. [33] noticed significant reduction in leaf RWC in okra and tomato with imposing drought tolerance. When RWC can be maintained in cells and tissues, it allows continuation of the metabolic activity by osmotic adjustment and other traits of adaptation to drought. Recovery of RWC after re-watering is a very important factor and often neglected in drought tolerant studies [5]. In faba bean, determination of leaf water potential was useful for describing the drought effect, but was not suitable for discriminating tolerant from sensitive genotypes. This suggested that water potential was not the defining feature of the tolerance [34]. Nevertheless, other studies opined that determination of leaf water status in the morning and water content in leaves in the afternoon were potentially useful for screening drought tolerance in chickpea [35]. The RWC correlated indirectly with the activity of SOD, GR and APX, arein *A. hypochondriacus* decreased to a very low 33% after 17 days of severe drought stress [5]. Similarly a sharp decline in RWC below 30% was experienced several Bermuda grass *spp* [36], below 40% in spruce sp. and 45% in maize [37] during severe drought stress.

3.5 Water Use Efficiency

Water use efficiency is an important indicator for plant adaptation and resistance to drought conditions [38]. Water use efficiency (WUE) is traditionally defined either as the ratio of dry

matter accumulation to water consumption over a season; or as the ratio of photosynthesis to transpiration over a period of time. It is among one of traits that has been studied extensively because it can give an idea of the variation amongst genotypes in their ability to utilize water efficiently under limited water supply [39]. Comprehensive literature on crop WUE based on carbon isotope dissemination studies depicts relation of WUE and drought tolerance with yield potential. Briefly, apparent genotypic variations in WUE are expressed mainly due to variations in water use. Higher WUE is generally achieved by specific plant traits and environmental responses that reduce yield potential in tomato [40]. Under most dryland situations where crops rely on unpredictable seasonal rainfall, the maximization of soil moisture use is a crucial component of drought resistance (avoidance), which is generally expressed in lower WUE [41]. It is now well documented that high yield potential and high yield under water-limited conditions is generally associated with reduced WUE mainly because of high water use [25].

3.6 Osmotic Adjustment

Osmotic adjustment (OA) has been considered as an important physiological adaptation associated with drought tolerance and has drawn substantial attention during the past years. Osmotic adjustment is defined as the active accumulation of organic solutes in the plant tissue in response to an increasing water deficit. By means of osmotic adjustment, the organelles and cytoplasmic activities take place at about a normal pace and help plants to perform better in terms of growth, photosynthesis and assimilate partitioning [4,16]. It is considered as a useful process for maintaining cell turgor when tissue water potential declines. OA has been shown to maintain stomatal conductance and photosynthesis at lower water potentials, delayed leaf senescence and death, reduced flower abortion, improved root growth and increased water extraction from the soil as water deficit develops [42]. OA involves the net accumulation of solutes in a cell in response to fall in water potential of the cell's environment. As a consequence, the cell's osmotic potential is diminished which in turn attracts water into the cell by tending to maintain turgor pressure. According to Martinez-Ballesta et al. [43] compatible solutes like sugars, glycerol, amino acids such as proline or glycinebetaine, polyols, sugar alcohols (like mannitol and other low molecular weight metabolites) would also

contribute to this process. In addition, Hessini et al. [44] argued that these compounds benefit stressed cells in two ways: either by acting as cytoplasmic osmolytes, thereby facilitating water uptake and retention and or by protecting and stabilizing macromolecules and structures (i.e. proteins, membranes, chloroplasts, and liposomes) from damage induced by stress conditions. Physiological indices such as leaf water potential, solute potential, relative water content, turgor potential, osmotic adjustment, leaf diffusive conductance (KI), difference between canopy and air temperature and water loss from excised leaves can be used as a screening tools. Improved tissue water status may be achieved through osmotic adjustment and/or changes in cell wall elasticity. This is essential for maintaining physiological activity for extended periods of drought [45]. Wild melon plant survived drought by maintaining its water content without wilting of leaves even under severe drought. Drought stress in combination with strong light led to an accumulation of high concentrations of citrulline, glutamate and arginine in leaves of wild watermelon. The accumulation of citrulline and arginine may be related to the induction of dopamine receptor interacting protein gene-1, a homologue of the acetyl ornithine deacetylase gene in *Escherichia coli*, where it functions to incorporate the carbon skeleton of glutamate into the urea cycle [46]. It has identified that among various mechanisms, osmotic adjustment, synthesis abscisic acid and induction of dehydrins may confer tolerance against drought injuries by maintaining high tissue water potential. The osmotic adjustment also facilitates a better translocation of pre-anthesis carbohydrate partitioning during grain filling, while high turgor maintenance leads to higher photosynthetic rate and growth [47].

3.7 Proline

Proline is generally considered as a good indicator of environmental stress and there are many reports describing an increase in proline content in response to water stress [17,18]. Ghorbanli et al. [18] suggested that Proline is one of the protective molecules that can unite oxygen and free radicals caused by stress. Therefore, a significant role of proline is probably reacting against drought stress [48]. Since proline plays a role as an osmotic factor, low water stress increased the proline content in plant. A relatively recent study on tomato found that use of brasinoestroid in two stress levels (mild and severe) increased the amount of

proline (3 and 4 respectively) in comparison with control. The effect of drought stress was also investigated on ABA (abscisic acid) and proline in different *Zea mays* species and a close correlation was established between proline accumulation and ABA with drought stress [49].

According to Abdalla and El-Khoshiban [9] free proline may be acting as a storage compound for carbon and nitrogen during drought stress when both starch and protein synthesis are inhibited. Such a storage compound might be utilized for growth upon rewatering, and after rewatering the enhanced level of proline decreases rapidly [50]. It is possible that both proline accumulation and antioxidative enzyme activities could be used as an index of drought tolerance [5,10]. The higher proline accumulation accompanied by higher enzyme activities of SOD, APX and CAT could suggest that the antioxidative defense mechanism is activated by increased proline production [5,10]. Proline acts as an osmolyte beside enzymes and other macromolecules, and therefore, protects the plant against low water potential and causes osmotic regulation in plant organs [18,48]. Also proline can act as an electron receptor preventing photosystem injuries in dealing with ROS function. Proline accumulation facilitates the permanent synthesis of soluble substances in closing stomata.

3.8 Soluble Sugars

The accumulation of soluble sugars (sucrose, glucose and fructose) is strongly correlated to the acquisition of drought tolerance in plants [51]. It is well known that sugars protect the cells during drought by two mechanisms. First, the hydroxyl groups of sugars may substitute for water to maintain hydrophilic interactions in membranes and proteins during dehydration. Hence, sugars interact with proteins and membranes through hydrogen-bonding, thus preventing protein denaturation. Secondly, sugars are a major contributing factor to vitrification, which is the formation of a biological glass in the cytoplasm of dehydrated cells. These intracellular glasses, by virtue of their high viscosity, drastically reduce molecular movement, impede the diffusion of reactive compounds in the cell, and may maintain the structural and functional integrity of macromolecules. It is by this property that glasses are thought to prolong the longevity of desiccated tissues by slowing down degradation processes during storage [17]. Mutava et al. [52] reported that fructose accounted for much of the significant increase in leaf total sugars under drought stress. Some studies have pointed out

that soluble sugar changes do not follow a static model and may vary with the genotype and the stress factor [53]. Gupta and Kaur [54] suggested that sucrose and glucose either act as substrates for cellular respiration or osmolytes to maintain cell homeostasis while fructose is involved in the synthesis of secondary metabolites as well as erythrose-4-P, which act's as a substrate in lignin and phenolic compound synthesis [55]. This suggests that under stress conditions the metabolism of soluble sugars is a dynamic process involving simultaneously degradation and synthetic reactions [17,52]. Soluble sugars, especially sucrose, accumulate in seeds, pollen and in drought-tolerant vegetative tissues. In many higher plants under dehydration stress, carbohydrate metabolism is shifted to favour the conversion of other sugars to sucrose. Trehalose, a non-reducing sugar, is also a potential organic osmolyte which has a substantial role in the protection of plants against drought stresses [56].

3.9 Reactive Oxygen Species (ROS)

ROS are partially reduced forms of atmospheric oxygen. They typically result from the excitation of O_2 to form singlet oxygen (O_2^1) or from the transfer of 1, 2 or 3 electrons to O_2 , for superoxide radical, hydrogen peroxide H_2O_2 or a hydroxyl radical (OH^\bullet), respectively. The cells are normally protected against ROS by the operation of the antioxidant defense system comprising enzymatic and non-enzymic components. The activities of enzymes of the antioxidant system in plants under stress are usually regarded as an indicator of the tolerance of genotypes against stress conditions. Overall, the involvement of ROS in various metabolic processes in plant cells might have general implications. Drought stress enhances the production of ROS in cellular compartments such as chloroplasts, peroxisome and mitochondria. ROS causes the peroxidation of membrane lipids, the denaturation of proteins and damage to nucleic acids [57]. If drought stress is prolonged, ROS productions will un-date the scavenging action of the antioxidant system, resulting in extensive cellular damage and eventual death. ROS are highly deleterious by-products of stress, and are likely to be important secondary messengers that trigger adaptation responses to the changing environment [58]. Drought stress induces the formation of active oxygen species by misdirection of electrons in the true photosystem.

3.10 Antioxidant

The antioxidant defense system in the plant cell constitutes both enzymatic and non-enzymatic components. Non-enzymatic components include β -carotene, ascorbate (ASC), α -tocopherol, reduced glutathione (GSH), carotenoid, enzymes include superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), polyphenol oxidase (PPO) and glutathione reductase (GR) [59]. In environmental stress tolerance, such as drought, high activities of antioxidant enzymes and high content of non-enzymatic constituents are important. The reactive oxygen species in plants are removed by a variety of antioxidant enzymes and lipid-soluble and water soluble scavenging molecules, the antioxidant enzymes being the most efficient mechanism against oxidative stress [35]. Apart from catalase, various peroxidases and peroxiredoxins, four enzymes are involved in the ascorbate-glutathione cycle, a pathway that allows the scavenging of superoxide radicals and H_2O_2 . These include ascorbate peroxidase, dehydroascorbate reductase (DHAC), monodehydroascorbate reductase and glutathione reductase [60]. The ascorbate glutathione cycle enzymes are located in the cytosol, stroma of chloroplasts, mitochondria and peroxisomes. Ascorbate peroxidase is the key antioxidant enzyme in plants whilst glutathione reductase has a central role in maintaining the reduced glutathione pool during stress [61]. Two glutathione reductase complementary deoxyribonucleic acids have been isolated; type one encoding the cytosolic isoforms [62] and the other encoding glutathione reductase proteins dual-targeted to both chloroplasts and mitochondria in different plants [63]. The capability of antioxidant enzymes to scavenge ROS and reduce the damaging effects may correlate with the drought resistance of plants [4]. The transcript of some of the antioxidant genes such as glutathione reductase or ascorbate peroxidase was higher during recovery from a water deficit period and appeared to play a role in the protection of cellular machinery against damage by reactive oxygen species [16].

3.11 Carotenoids

Carotenoids a tetra-terpenoids are pigments with several functions in plants, besides their direct role in photosynthesis, they are actively involved in the mechanisms of oxidative stress tolerance. Carotenoids are a large class of isoprenoid molecules, which are synthesized by all photosynthetic and many non-photosynthetic

organisms. They are divided into the hydrocarbon carotenes, such as lycopene, α -carotene and β -carotene, and xanthophylls, typified by lutein. Carotenes form a key part of the plant antioxidant defense system, but they are very susceptible to oxidative destruction. β -carotene, present in the chloroplasts of all green plants is exclusively bound to the core complexes of PSI and PSII. Protection against damaging effects of ROS at this site is essential for chloroplast functioning. In addition to the function at an accessory pigment, β -carotene acts as an effective antioxidant and plays a unique role in protecting photochemical processes and sustaining them. A major protective role of β -carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and protects from oxidative damage [16]. Unyayar et al. [64] observed decreased chlorophyll *a/b* ratio in drought tolerant *Lycopersicon peruvianum* and concluded that this trait indicates better protection of PSII against drought stress. An increase in carotenoid/chlorophyll ratio might be of a protective value as carotenoids are known to be potent quenchers of ROS, particularly singlet oxygen. Carotenoids and other compounds, such as abietane diterpene, have received little attention despite their capacity to scavenge singlet oxygen and lipid peroxy-radicals, as well as to inhibit lipid peroxidation and superoxide generation under dehydrative forces [65].

3.12 Tocopherols

Tocopherol, a lipid soluble antioxidant is considered as a potential scavenger of ROS and lipid radicals [66]. Tocopherols are considered as major antioxidant in biomembranes, where they play both antioxidant and non-antioxidant functions. Tocopherols are considered general antioxidants for protection of membrane stability, including quenching or scavenging ROS [67]. They are localized in plants in the thylakoid membrane of chloroplasts. Out of four isomers of tocopherols found in plants, α -tocopherol has the highest antioxidative activity due to the presence of three methyl groups in its molecular structure. Increased levels of α -tocopherol and ASH have been found in tomato following trizole treatment which may help in protecting membranes from oxidative damage [68].

3.13 Glutathione (GSH)

Glutathione (GSH, γ -glutamyl-cysteinyl-glycine) is a low molecular weight thiol-metabolite and a

major antioxidant in plant cells. Glutathione is present in most eukaryotic organisms, where it performs multiple functions. These include storage and transport of sulphur and control of the redox status. GSH is a strong reductant that can scavenge ROS directly or in cooperation with other antioxidants and ROS processing enzymes. It seems likely that GSH levels are used as a cue in the coordination of mechanisms for the supply of cysteine and the maintenance of the cellular NADPH pool. Coordination of these responses, merely by lowering the GSH pool, provides plants with a simple mechanism to respond defensively to a wide range of stresses through a coordinated up-regulation of the capacity for GSH biosynthesis and its redox cycling [5].

3.14 Catalase

Plant catalases are tetrameric iron porphyrins and play a role in stress tolerance against oxidative stress. Catalases are produced in peroxisomes and glyoxysomes. These are involved in eliminating hydrogen peroxide generated by different environmental stresses [69,70]. Catalase decomposes hydrogen peroxide to water and molecular oxygen without consuming reductants and thus may provide plant cells with an energy efficient mechanism to remove hydrogen peroxide [70]. The enzyme is abundant in the glyoxysomes of lipid-storing tissues in germinating barley, where it decomposes H_2O_2 formed during the oxidation of fatty acids and in the peroxisomes of the leaves of C_3 plants, where it removes H_2O_2 generated during photorespiration by the conversion of glycolate into glyoxylate. This is also due to the fact that there is a proliferation of peroxisomes during stress, which might help in scavenging H_2O_2 [71].

3.15 Glutathione Reductases (GR)

GR is a flavo-protein oxidoreductase, found in both prokaryotes and eukaryotes [72]. It is a potential enzyme of the ASH-GSH cycle and plays an essential role in defense system against ROS by sustaining the reduced status of GSH. It is localized predominantly in chloroplasts, but small amount of this enzyme has also been found in mitochondria and cytosol. GR catalyzes the reduction of glutathione (GSH), a molecule involved in many metabolic regulatory and antioxidative processes in plants, e.g., GR catalyses the NADPH dependent reaction of disulphide bond of oxidized glutathione (GSSG)

and is thus, important for maintaining the GSH pool [73]. Actually, GSSG consists of two GSH molecules linked by a disulphide bridge which can be converted back to GSH by GR. GR is involved in defense against oxidative stress, whereas, GSH plays an important role within the cell system including participation in the ASH-GSH cycle, maintenance of the sulfhydryl (eSH) group and a substrate for glutathione transferases (GSTs) [74]. It was suggested that GR plays an important role in the regeneration of GSH and thus protects against oxidative stress by maintaining the ASH pool [75].

3.16 Malondialdehyde (MDA)

Accumulation of MDA, mainly produced from the ROS induced degradation of membrane lipids, is a potential biomarker to assess the severity of the abiotic stress, including drought stress [76]. Membrane damage is sometimes taken as a single parameter to determine the level of lipid destruction under various stresses. Now, it has been recognized that during lipid peroxidation products are formed from polyunsaturated precursors that include small hydrocarbon fragments such as ketones, MDA, etc and their related compounds [77]. MDA is synthesized due to degradation of polyunsaturated lipids by ROS. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress. Increased MDA accumulation has been correlated with reduction of relative water content and photosynthetic pigment content under prolonged drought. Lower MDA displays higher anti-oxidative ability, reflecting higher resistance to drought. These secondary metabolites play multiple roles in plants, including scavenging of ROS induced under different stress conditions and causing oxidative stress [78,79]. A clear symptom of oxidative damage is cell membrane degradation; therefore, MDA - a product of membrane lipid peroxidation is an excellent marker of oxidative stress [77]. A significant increase of MDA level in tomato leaves upon induced drought stress was observed [17].

4. CONCLUSION

As climate prediction models predict increased manifestation of drought, salinity, high and low temperature spells during the crop growth periods, global food production will continue to be challenged. Ensured sustainable yields under changing environmental conditions will therefore be imperative. Prolonged soil water deficit causing drought, is one of the prevalent abiotic

stresses vastly responsible for the production of ROS in different cellular compartments like chloroplasts, mitochondria, peroxisomes, etc. which further attack biomolecules, viz., DNA, lipids, proteins, carbohydrates, thereby disturb the normal functioning of the cell. Different physiological mechanisms including stomatal conductance, osmotic adjustment, , water use efficiency, cell membrane stability and relative water content make it possible for the plant to tolerate the damage caused by drought stress. In order to withstand oxidative stress, plants are equipped with enzymatic and nonenzymatic antioxidants. Non-enzymatic (ascorbate, carotenoids and tocopherols) and enzymatic antioxidants (Catalase, glutathione, glutathione reductase, SOD, APX, etc.) have a predominant role in combating oxidative damage of the cell. To overcome the deleterious effects of abiotic stress, plants accumulate osmolytes and osmoprotectants, particularly proline and glycine betaine. These compounds are thought to play a significant role in osmotic adjustment and protection of subcellular structures thereby enhancing drought tolerance of plants. Manipulation of traits conferring drought tolerance can be exploited for sustaining qualitative and quantitative production in the condition of water scarcity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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